ISSN (print) 0093-4666 © 2012. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/119.1

*Volume 119, pp. 1–10* *January–March 2012*

# Four new species of *Diorygma* from India

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Abstract—Four new species of the lichen genus *Diorygma* from India are described — *D. agumbense, D. dandeliense,* and *D. karnatakense* from the Western Ghats and *D. rupicola* from Northeast India.

Keywords—ascomycetes, taxonomy, *Ostropales, Graphidaceae*

## Introduction

The lichen genus *Diorygma* Eschw. is widespread in tropical and subtropical regions and c. 45 species are known from around the world (Kalb et al. 2004, Archer 2006, 2007, Archer & Elix 2008, Cáceres 2007, Sharma & Makhija 2009a,b, Makhija et al. 2009, Tripp et al. 2010), 21 of which are known from India. The main characters that define *Diorygma* are lirellate ascocarps with a heavily pruinose disc, parallel paraphyses with thin lumina, thick gelatinous walls, and tips that anastomose to form an epithecium, *Graphis*-type asci, and hyaline to (rarely) brownish trans-septate or muriform ascospores. Kalb et al. (2004), who monographed the genus, and confirmed its placement within *Graphidaceae* through molecular phylogenetic analysis.

The combination of norstictic and salazinic acids is very rare in *Diorygma*. In our previous studies on *Graphidaceae* from India, we proposed four new *Diorygma* species with norstictic and salazinic acids as major compounds: *D. dealbatum* B.O. Sharma & Makhija, *D. inaequale* B.O. Sharma & Makhija, *D. manipurense* B.O. Sharma & Makhija, and *D. verrucirimosum* B.O. Sharma & Makhija (Sharma & Makhija 2009a). Since then, we have encountered four additional new species of *Diorygma,* which are described here. Two — *D. dandeliense* and *D. karnatakense* — contain only norstictic and salazinic acids, while *D. agumbense* (with protocetraric acid) and the saxicolous *D. rupicola* are also new to science. A key to the identification of *Diorygma* species in India is provided.

## Materials & methods

Approximately 80 specimens representing four *Diorygma* species were collected from different regions of India Western Ghats —Karnataka, Northeast India —Meghalaya. Their external morphology was studied using a Zeiss dissecting microscope; sections for anatomical studies were stained with Lugol's iodine, mounted in lactophenol, and examined with a Zeiss Axioskop microscope. Images were captured using a Nikon Coolpix 8400 digital camera.

The specimens were identified by comparison with protologues or descriptions and photographs of known species in the most recent treatments (Kalb et al. 2004, Archer 2006, 2007, Archer & Elix 2008, Makhija et al. 2009; Sharma & Makhija 2009a,b).

Chemical constituents were identified by thin-layer chromatography using methods standardized for lichen products (Culberson & Kristinsson 1970, Culberson 1972, White & James 1985) with the solvent systems benzene-dioxane-acetic acid (180:45:5), hexane-ethyl ether-formic acid (130:80:20), and toluene-ethyl acetate-formic acid (139:83:8). All examined specimens are deposited in Ajrekar Mycological Herbarium (AMH).

## Taxonomy

***Diorygma agumbense*** B.O. Sharma & Khadilkar, **sp. nov.** Fig. 1

MycoBank MB 561545

*Species nova distincte inter* Diorygma *specibus ob continentem acidum protocetraricum sed ascosporis 8-nae differt.*

Type: India. Karnataka: Hebri, Agumbe-Udipi road, 16.12.1974, Patwardhan & A.V. Prabhu (**Holotype**, AMH 74.3041).

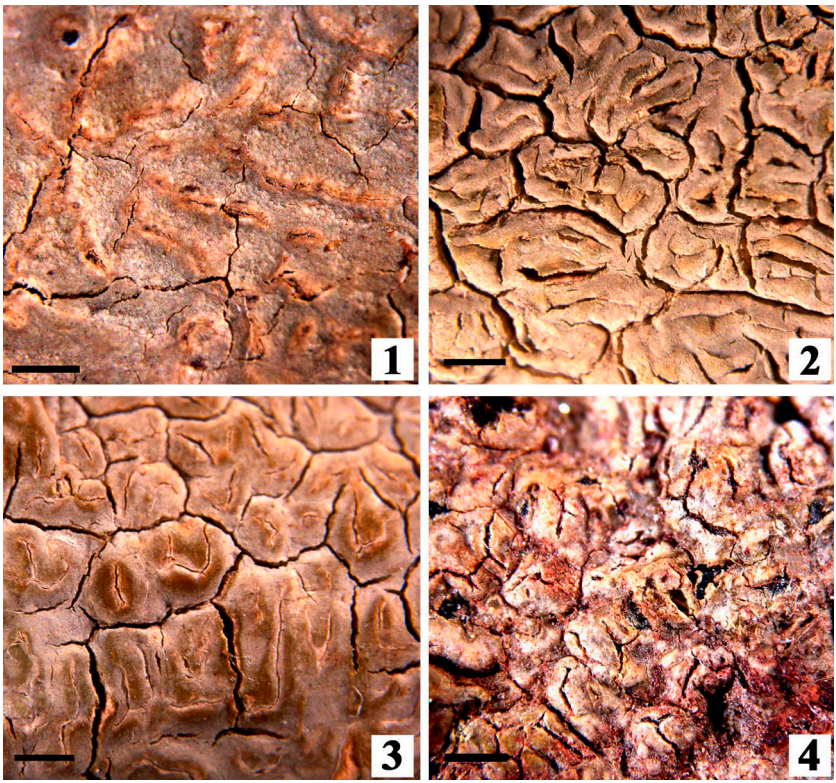
Etymology: Referring to Agumbe, the collecting locality

Thallus corticolous, pale greenish-grey, rough, cracked, delimited by thin, black hypothallus. Ascocarps lirelline, concolorous with the thallus, 1–1.5 mm long, mostly simple, rarely branched, slightly raised to mostly immersed. Disc narrow, slit-like, rarely ± open, covered with white pruina. Exciple convergent, non-carbonized, non-striate. Hymenium hyaline, laterally KI+ blue, clear, not inspersed. Asci 8-spored, hyaline, muriform, I+ blue violet, peripheral and central locules of more or less equal size, regularly arranged in rows, 87–125 × 20–37 μm.

Chemistry—Cryptostictic, protocetraric, and stictic acids present (K+ yellow, P+ red).

Ecology & distribution— in semi-evergreen forest in open places on the roadsides. Known only from the type locality.

Remarks—*Diorygma africanum* Kalb et al., *D. hololeucum* (Mont.) Kalb et al., and *D. pruinosum* (Eschw.) Kalb et al., the only other *Diorygma* species known to have protocetraric acid as a major compound, differ in having a distinctly divergent exciple, 1–2-spored asci, and an absence of stictic acid. The combination of protocetraric and stictic acid complex is rather unique in the genus and no species previously has been reported with this chemistry.




Figures 1–4. Habit: 1. *Diorygma agumbense* (Holotype AMH 74.3041); 2. *D. dandeliense* (Holotype AMH 04.276); 3. *D. karnatakense* (Holotype AMH 04.280); 4. *D. rupicola* (Holotype AMH 77.779). Scale bars = 1 mm.

*Diorygma erythrellum* (Mont.) Kalb et al. and *D. minisporum* Kalb et al. both have 8-spored asci but differ from *D. agumbense* in small ascospores and chemistry. *Diorygma erythrellum* has ascospores of 30–65 × 12–20 µm and produces norstictic acid, whereas *D. minisporum* has transversely septate ascospores of 17–20 × 5–6.5 µm and lacks lichen substances.

***Diorygma dandeliense*** B.O. Sharma & Khadilkar, **sp. nov.** Fig. 2

MycoBank MB 561546

*Similis* Diorygma dealbatum *sed ascospois majoribus et excipulum convergentum differt.*

Type: India, Karnataka, Dandeli forest, 5.10.2004, U.V. Makhija (**Holotype**, AMH 04.276).

Etymology: Referring to Dandeli, the collecting locality

Thallus corticolous, pale greenish-grey, smooth. Ascocarps lirelline, concolorous with the thallus, 0.5–2 mm long, mostly simple, rarely branched, curved, slightly raised to immersed. Disc narrow, slit-like, rarely ± open, covered with white pruina. Exciple convergent, non-carbonized, non-striate. Hymenium hyaline, laterally KI+ blue, clear, not inspersed. Asci 1-spored, hyaline, muriform, I+ blue violet, peripheral and central locules of more or less equal size, regularly arranged in rows, 125–175 × 27–35 µm.

Chemistry—Norstictic and salazinic acids present (K+ yellow forming red crystals).

Additional specimens examined—**INDIA. Karnataka:** Dandeli forest, 5.10.2004, U.V. Makhija, AMH 04.277; B.C. Behera, AMH 04.269.

Remarks—*Diorygma dandeliense* shares a similar morphology and chemistry with *D. dealbatum,* which can be differentiated by its shorter (105–147 µm) ascospores and a divergent exciple.

The new species is very similar to *D. megasporum* Kalb et al. in morphology and ascospore range. However, the asci of *D. megasporum* contain 2–6 ascospores and the thallus lacks salazinic acid.

Both *D. excipuloconvergentum* Makhija et al. and *D. albocinerascens* Makhija et al., which are morphologically and chemically very close to *D. dandeliense,* have long (≤ 6 mm) lirellae, distinctly striate exciple, and 1–2-spored asci.

*Diorygma salvadoriense* Kalb et al. also contains norstictic and salazinic acids but differs from the new species in having distinctly divergent exciple and a carbonaceous basal layer.

***Diorygma karnatakense*** B.O. Sharma & Khadilkar, **sp. nov.** Fig. 3

MycoBank MB 561547

*Similis* Diorygma salvadoriense *sed peripheralis et centralis sporae loculis equalis differt.*

Type: India, Karnataka, Dandeli forest, 5.10.2004, U.V. Makhija (**Holotype**, AMH 04.280).

Etymology: From the state name, Karnataka.

Thallus corticolous, epiphloeodal, greenish-grey, dull, cracked, smooth. Ascocarps lirelline, concolorous with the thallus, 0.5–2 mm long, simple, curved, immersed, ends acute or rounded. Disc narrow, slit-like, covered with white pruina. Exciple convergent, non-carbonized, non striate. Hymenium hyaline, laterally KI+ blue, clear, not inspersed. Asci 1–4-spored, hyaline, muriform, I+ blue violet, peripheral and central locules of more or less equal size, 130–200 × 32–42 µm.

Chemistry— Norstictic and salazinic acids present (K+ yellow, forming red crystals).

Additional specimens examined—**INDIA**. **Karnataka**: Dandeli forest, 5.10.2004, U.V. Makhija, AMH 04.266, 04.441, 04.291.

Remarks—*Diorygma karnatakense* is close to *D. salvadoriense, D. reniforme* (Fée) Kalb et al., *D. rufopruinosum* (A.W. Archer) Kalb et al., *D. albocinerascens,* and *D. excipuloconvergentum* in having large ascospores and in containing norstictic and salazinic acids as major substances. However, *D. salvadoriense, D. rufopruinosum,* and *D. reniforme* do not produce ascospores with the same-sized peripheral and central cells. Moreover, *D. salvadoriense* and *D. reniforme* have a distinctly divergent, orange brown to basally carbonized, entire exciple.

*Diorygma albocinerascens* and *D. excipuloconvergentum* from Maharashtra, India, also containing norstictic and salazinic acids and morphologically similar, differ from *D. karnatakense* in a distinctly striate exciple.

***Diorygma rupicola*** B.O. Sharma & Makhija, **sp. nov.** Fig. 4

MycoBank MB 561548

*Similis* Diorygma hieroglyphicum *sed habitus saxicola et excipulum convergentum differt.*

Type: India, Meghalaya, Mawrong, on Cherrapunjee Road, 27.10.1977, P.G. Patwardhan & M.B. Nagarkar (**Holotype**, AMH 77.779).

Etymology: From the Latin *rupes*, rock, + *–cola*, dweller, a reference to the species habit on rock.

Thallus saxicolous, grey-brown colored. Ascocarps concolorous with the thallus, short, 0.2–0.5 mm long, simple, or irregularly branched, curved, immersed. Disc narrow, rarely ± open, pruinose. Exciple convergent, apically 1–2 striate, carbonized at the tips. Hymenium hyaline, laterally KI+ blue, clear, not inspersed. Asci 1–2-spored, hyaline, muriform, I+ blue violet, peripheral and central locules of more or less equal size, regularly arranged in rows, 88–147 × 25–42 µm.

Chemistry—Constictic, cryptostictic, and stictic (all major) and norstictic (minor) present (K+ yellow).

Ecology & distribution— collected from the tropical and sub-tropical regions of Meghalaya, Nagaland and Sikkim at ~1500–2000 m.

Additional specimens examined—**INDIA. Meghalaya:** Wiloe, 30.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.1092; Cherrapunjee Road, 27.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.816, 77.817; Mohmtheid-Cherrapunjee road, 28.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.1027, 77.1031, 77.1040; Mawrong, on Cherrapunjee Road, 27.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.2048; Mawsynram, 30.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.1135; Nagaland, 12 km to Zabuza, 7.11.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.1468; Sikkim, near Tangshi view point, Gangtok, 17.10.77, P.G. Patwardhan & M.B. Nagarkar, AMH 77.1947.

Remarks—*Diorygma rupicola* is morphologically very close to *D. hieroglyphicum* (Pers.) Staiger & Kalb, which differs in having a corticolous habit, divergent exciple, and ascospores with a gelatinous sheath. *Diorygma albocinerascens*, a corticolous species with similar exciple morphology and

spore size, can easily be distinguished from the new species in its much narrower (18–27 µm) ascospores and cryptostictic, methylstictic, norstictic and salazinic acids (all major).

*Diorygma monophorum* (Nyl.) Kalb et al., resembles *D. rupicola* in exciple characters, 1–2-spored asci, large and very broad ascospores (105–165 × 35–60 µm), but it differs in having hypostictic, hypoconstictic, and α-acetytylhypoconstictic acids (major or submajor).

### Key to the species of *Diorygma* from India

1a. Ascospores exceeding 200 µm in length ..... 2

1b. Ascospores not exceeding 200 µm in length ..... 5

2a. Exciple distinctly divergent; disc exposed; thallus grayish white, powdery, smooth; ascocarps concolorous with the thallus, 1–3 mm long, emergent; disc yellowish to brownish; exciple divergent, non-carbonized; asci 1-spored; ascospores muriform, (135–)168–205 × 54–96 µm with 5–7.5 µm thick sheath; constictic (major) and stictic (major) acids present ..... ***D. rufosporum*** (Patw. & C.R. Kulk.) B.O. Sharma & Makhija

2b. Exciple distinctly convergent; disc hidden ..... 3

3a. Norstictic acid absent; thallus corticolous, greenish white; ascocarps 0.5–9 mm long and 0.1–0.25 mm broad, simple, rarely branched, disc narrow, pruinose; exciple convergent, 2–6-striate, blackish brown at apices and dark brown laterally; asci 1-spored; ascospores muriform, 231–244 × 59–76 µm, consalazinic, constictic, cryptostictic, and stictic acids (major) present.... ***D. megaspermum*** Makhija et al.

3b. Norstictic acid present ..... 4

4a. Thallus corticolous, glaucous, whitish to greenish gray; ascocarps 1–4 mm long simple to rarely branched, whitish, or concolorous with the thallus; disc slit like or broader, blackish brown to black, white pruinose when open; exciple poorly developed, 3–4 indistinct striation, blackish brown at apices, pale yellow to almost hyaline, convergent; asci 1–4-spored; ascospores muriform, 151–294 × 38–63(–84) µm, cryptostictic, norstictic, and stictic acids present ..... ***D. megistosporum*** Makhija et al.

4b. Thallus corticolous, smooth to cracked, pale glaucous green to olivaceous buff or whitish; ascocarps 1–6 mm long simple to rarely branched, immersed to semi-emergent, concolorous with the thallus; disc white, slit-like, later moderately open, white pruinose; exciple poorly developed, pale yellow, indistinctly 2–4-striate, blackish brown at apices; ascospores 1–2–4/ascus, 147–273 × 34–67 µm; constictic, cryptostictic, methylstictic, norstictic, and salazinic acids present ..... ***D. excipuloconvergentum***

5a. Ascospores exceeding 100 µm in length ..... 6

5b. Ascospores not exceeding 100 µm in length ..... 20

6a. Thallus saxicolous ..... 7

6b. Thallus corticolous ..... 8

7a. Thallus saxicolous, grey-brown colored; ascocarps concolorous with the thallus, short, 0.2–0.5 mm long, simple, or irregularly branched; exciple convergent, apically 1–2 striate, carbonized at the tips; asci 1–2-spored, muriform, 88–147 × 25–42 µm; constictic, cryptostictic, stictic (major), and norstictic (minor) present ..... ***D. rupicola***

7b. Thallus saxicolous, greenish to pale gray, rough; ascocarps concolorous, curved, short, round to elongate, simple to branched, very closely arranged, immersed to slightly emergent; disc narrow to broad; exciple divergent, non-carbonized, poorly developed, yellowish brown laterally and basally, 2–3 indistinct striate at the apical region; asci 1–6-spored; ascospores muriform, 143–172 × 29–34 µm; constictic, norstictic (trace) and stictic acids present ..... ***D. saxicola*** B.O. Sharma & Makhija

8a. Protocetraric acid present ..... 9

8b. Protocetraric acid absent ..... 10

9a. Thallus corticolous, whitish green; ascocarps concolorous with the thallus, circular, oval, short curved, 1–3 mm long; disc grayish, broad; exciple divergent, non-carbonized to carbonization sometimes restricted to the basal corners; asci 1-spored; ascospores muriform, 147–160 × 34–42 µm; protocetraric acid ..... ***D. pruinosum***

9b. Thallus corticolous, pale greenish-grey; ascocarps lirelline, concolorous with the thallus, 1–1.5 mm long, mostly simple, rarely branched; disc narrow, covered with white pruina; exciple convergent, non striate; asci 8-spored, muriform, 87.5–125 × 20–37.5 µm; cryptostictic, protocetraric and stictic acids present ..... ***D. agumbense***

10a. Asci 8-spored ..... 11

10b. Asci more than 1-spored ..... 12

11a. Thallus corticolous; exciple convergent; ascospores 1–8/ascus, 85–197 × 29–71 µm; constictic, stictic, and cryptostictic acids present. ..... ***D. megasporum***

11b. Thallus corticolous, ashy white; ascocarps white, straight to curved, branched, 0.5–2.5 mm long, immersed; disc 0.3–0.6 mm broad; exciple convergent to divergent, indistinctly striate; asci 1–8-spored; ascospores 75–145 × 24–33.6 µm; norstictic and stictic acids present ..... ***D. subalbatum*** (Patw. & Makhija) B.O. Sharma & Makhija

12a. Exciple distinctly convergent; disc hidden ..... 13

12b. Exciple distinctly divergent; disc exposed ..... 16

13a. Asci 1-spored ..... 14

13b. Asci more than 1-spored ..... 15

14a. Thallus corticolous, pale greenish-grey; ascocarps lirelline, concolorous with the thallus, 0.5–2 mm long; disc narrow, covered with white pruina; exciple convergent, non striate; asci 1-spored, muriform, 125–175 × 27.5–35 µm; norstictic and salazinic acids present..... ***D. dandeliense***

14b. Thallus corticolous, greenish grey with red tinge; ascocarps long flexuous, branched, curved, up to 9 mm long; disc slightly open, pale brown; exciple

convergent to divergent, non-carbonized; asci 1-spored; ascospores muriform, 105–113 × 33–42 µm; norstictic acid present
. . . . . ***D. longilirellatum*** B.O. Sharma & Makhija

15a. Thallus corticolous, whitish gray, finely cracked; ascocarps 0.5–6 mm long simple, dendroidly branched, immersed, straight or curved, concolorous with the thallus; disc narrow, slit-like, light brown, slightly pruinose; exciple convergent, 2–3-striate, blackish brown at apices, asci 1–2-spored; ascospores 135–150 × 18–27 µm; cryptostictic, methylstictic, norstictic, and salazinic acids present
. . . . . ***D. albocinerascens***

15b. Thallus corticolous, greenish-grey, dull; ascocarps concolorous with the thallus, 0.5–2 mm long, simple, curved, immersed; disc narrow, covered with white pruina; exciple convergent, non striate; asci 1–4-spored, muriform, 130–200 × 32.5–42.5 µm; norstictic and salazinic acids present . . . . . ***D. karnatakense***

16a. Norstictic and salazinic acids present . . . . . 17

16b. Salazinic acid absent . . . . . 18

17a. Thallus corticolous, glaucous grey, deeply cracked, distinctly verrucose; ascocarps crowded, concolorous with the thallus, irregular, short, 1–2 mm long; disc narrow to wide, sunken, 0.2–0.4 mm wide, reddish brown covered with white pruina; exciple, divergent, uncarbonized, base distinctly orange brown; asci 1-spored, muriform, 97–126 × 25–33 µm; norstictic and salazinic acids present . . . . . ***D. verrucirimosum***

17b. Thallus corticolous, white with brown tinge, smooth, thick; ascocarps rounded, lirellate, concolorous with the thallus, immersed, irregular, simple to branched, curved, 0.5–2.5 mm long; disc narrow to broad, 0.2–0.7 mm; exciple divergent, uncarbonized, laterally poorly developed, base distinctly orange brown; asci 1-spored, ascospores muriform, 105–147 × 33–37 µm; norstictic and salazinic acids . . . . . ***D. dealbatum***

18a. Peripheral spore locules distinctly smaller than the central ones. Thallus corticolous, rough; ascocarps 1–5 mm long, raised from the thallus; exciple non-carbonized, yellowish orange; asci 1-spored; ascospores muriform, 100–130 × 44–48 µm; norstictic and con-norstictic acids present
. . . . . ***D. tuberculosa*** (Stirt.) Kalb et al.

18b. Peripheral and central spore locules of equal size . . . . . 19

19a. Thallus corticolous, greenish with whitish tinge, smooth; ascocarps off white to concolorous with the thallus, immersed 1–4 mm long; disc broad, brown, 0.2–0.4 broad, pruinose; exciple non-carbonized, poorly developed, yellowish brown laterally and basally not striate; asci 1-spored; ascospores muriform, 80–131 × 42–52 µm, with a 5–15 µm thick sheath; stictic, constictic and norstictic acids present. . . . . ***D. hieroglyphicum***

19b. Thallus corticolous, creamy white, grayish; ascocarps 0.4–4 mm long, round to irregular, branched, immersed to slightly raised; disc brownish grey covered by yellowish cream pruina; exciple divergent, non-carbonized, orange yellow at the

base; asci 1(–2)-spored; ascospores 84–134.4 × 29.4–42 µm, with a 2.3–5 µm sheath; constictic acid (trace), norstictic, and stictic acid (major) present. . . . . ***D. junghuhnii*** (Mont. & Bosch) Kalb et al.

20a. Asci 1-spored . . . . . 21

20b. Asci more than 1-spored . . . . . 22

21a. Thallus corticolous, glaucous green with a yellow and or red tinge, uneven; ascocarps 1–6 mm long, simple to irregularly branched; disc brown, 0.2–0.3 mm broad; exciple non-striate, divergent, non carbonized, distinctly orange at the base; asci 1-spored; ascospores muriform, 79–96 × 29–33 µm; norstictic and salazinic acids present. . . . . ***D. inaequale***

21b. Thallus corticolous, grayish white, smooth to rough, deeply cracked and appearing areolate; ascocarps concolorous with the thallus, 1–1.5 mm long, simple to branched, grouped, immersed; exciple hyaline, poorly developed, 2–3 striate, pale brown laterally, blackish brown at apices, convergent; asci 1-spored; ascospores muriform; 57–96 × 24–36 µm; consalazinic, constictic, cryptostictic, and stictic acids (major); Maharashtra state, [AMH 04.13] . . . ***D. "patwardhanii***"

22a. Norstictic and salazinic acid present . . . . . 23

22b. Salazinic acid absent . . . . . 24

23a. Thallus corticolous, grayish, smooth to rough; ascocarps 1–2 mm long, simple, semi-emergent, concolorous with the thallus; exciple 2–3 striate, pale brown laterally, blackish brown at apices, convergent; asci 1–2-spored; ascospores muriform, 75–99 × 24–30 µm; norstictic, salazinic, and methylstictic acids present . . . . . ***D. panchganiense*** Makhija et al.

23b.Thallus corticolous, brownish white, ± smooth to rough; ascocarps concolorous with the thallus, 0.2–0.3 mm long mostly simple to rarely branched; disc brown, 0.4–0.5 mm broad; exciple 2–5 striate, apically dark brown, divergent; asci 2–4 spored; ascospores muriform, 76–92 × 21–25 µm; Norstictic and salazinic acids present . . . . . ***D. manipurense***

24a. Thallus corticolous, glaucous green, smooth to cracked; ascocarps 3–7 mm long simple to branched, immersed to emergent; disc reddish brown to blackish brown; exciple 2–5 striate, orange brown, carbonized at apices, convergent; asci 8-spored; ascospores muriform, 46–63 × 17–25 µm; stictic acid (major); Maharashtra state; [AMH 72.24] . . . . . ***D. "microsporum***"

24b. Thallus corticolous, greenish white, smooth to cracked; ascocarps 0.5–3 mm long simple to branched, concolorous with the thallus, fissure-like; exciple 2–3-striate, pale brown to blackish-brown at apices, convergent, asci 4–8-spored; ascospores muriform, 66–99 × 12–36 µm; constictic, cryptostictic, and stictic acids present . . . . . ***D. albovirescens*** Makhija et al.

(Note: species in quotations marks — *D. "patwardhanii"* and *D. "microsporum"* — are recorded by Makhija et al. (2009) and are probably new to science, but are not formally described due to scanty material.)

**Acknowledgments**

We are grateful to the Department of Science and Technology, Government of India, New Delhi for the financial support. The authors are grateful to A. Aptroot, J. Lendemer, and R. Lücking for reviewing the manuscript, critical revision, and suggestions to improve the quality of the manuscript.

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